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(54) Title: POLYMORPHISMS IN THE HUMAN HMG-COA REDUCTASE GENE

(57) Abstract: This invention relates to polymorphisms in the human HMG-CoA reductase gene and corresponding novel allelic polypepides encoded thereby. Particular polymorphisms are described in the promoter, exon (15) and introns (2, 5, 15) and (18). The invention also relates to methods and materials for analysing allelic variation in the HMG CoA reductance gene, and to the use of HMG-CoA reductase polymorphism in the diagnosis and treatment of HMG-CoA reductase mediated diseases such as dyslipidemia and other cardiovascular diseases such as myocardial infarction and stroke.

POLYMORPHISMS IN THE HUMAN HMG-COA REDUCTASE GENE

This invention relates to polymorphisms in the human HMG-CoA reductase gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the HMG CoA reductase gene, and to the use of HMG-CoA reductase polymorphism in the diagnosis and treatment of HMG-CoA reductase mediated diseases such as dyslipidemia and other cardiovascular diseases such as myocardial infarction and stroke.

At the time of priority filing, there were no known polymorphisms in the HMG-CoA reductase gene. On 7Oct1999, in PCT Application WO 99/50454, Lander *et al* published on a Ile to Val polymorphism at position 638 (see Figure 1B therein).

In the human HMG CoA reductase gene a single donor splice site is used to excise the intron in the 5' untranslated region. There are multiple mRNAs due to alternative start sites, all of which have short untranslated regions of 68 to 100 nucleotides ("Conservation of promoter sequence but not complex intron splicing pattern in human and hamster genes for 3-hydroxy-3-methylglutaryl coenzyme A reductase"; Mol. Cell. Biol. 7:1881-1893(1987).)

The HMG-CoA reductase gene has been cloned as cDNA and published as EMBL Accession number M11058 (2904 bp) as defined by SEQ ID NO 44. All positions herein of polymorphisms in the coding sequence relate to the position in SEQ ID NO 44 unless stated otherwise or apparent from the context. The protein sequence of the HMG-CoA reductase has also been been published in Luskey K.L. *et al* "Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. Conserved domains responsible for catalytic activity and sterol-regulated degradation"; J. Biol. Chem. **260**:10271-10277(1985).

A partial genomic sequence of HMG-CoA reductase, including the promoter and exon-1, is published as EMBL Accession number M15959 (1227 bp) as defined by SEQ ID NO 45 herein. All positions herein of polymorphisms in the promoter region relate to the position in SEQ ID NO 45 unless stated otherwise or apparent from the context.

All positions herein of polymorphisms in the intron regions relate to the position of the relevant intron sequence disclosed herein unless stated otherwise or apparent from the context.

HMG-CoA reductase is the rate-limiting enzyme for cholesterol synthesis and is regulated via a negative feedback mechanism mediated by sterols and non-sterol metabolites derived from mevalonate, the product of the reaction catalyzed by reductase. Normally in

mammalian cells, this enzyme is suppressed by cholesterol derived from the internalization and degradation of LDL via the LDL receptor. Competitive inhibitors (termed "statins") of the reductase induce the expression of LDL receptors in the liver, which in turn increases the catabolism of plasma LDL and lowers the plasma concentration of cholesterol, an important determinant of atherosclerosis.

The sequence coding for the highly conserved membrane bound region of the protein is located at positions 51-1067, that coding for the linker part of the protein at positions 1068-1397 and for the strongly conserved water-soluble catalytic part at positions 1398-2714.

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics") . Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, **43**, 254; Marshall (1997), Nature Biotechnology, **15**, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, **16**, 33.

Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

Point mutations in polypeptides will be referred to as follows: natural amino acid (using 1 or 3 letter nomenclature) , position, new amino acid. For (a hypothetical) example "D25K" or "Asp25Lys" means that at position 25 an aspartic acid (D) has been changed to lysine (K). Multiple mutations in one polypeptide will be shown between square brackets with individual mutations separated by commas.

The present invention is based on the discovery of the genomic structure of HMG-CoA reductase and polymorphism therein. In particular, we have found one single nucleotide polymorphism (SNP) in the coding sequence of the HMG-CoA reductase gene, 2 SNPs in the promoter sequence of the HMG-CoA reductase gene and 5 SNPs in the intron sequence of the HMG-CoA reductase gene as well as the genomic structure of the gene and novel sequence allowing the discovery of SNPs in the exons and introns of the gene.

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in HMG-CoA reductase in a human, which method comprises determining the sequence of the nucleic acid of the human at at least one polymorphic position and determining the status of the human by reference to polymorphism in the HMG-CoA reductase gene. Preferred polymorphic positions are one or more of the following positions:

position 1962 in the coding sequence of the HMG-CoA reductase gene as defined by the position in SEQ ID NO: 44, and/or

positions 46 or 267 in the promoter sequence of the HMG-CoA reductase gene as defined by the positions in SEQ ID NO: 45; and/or

position 129 in intron 2 as defined by the position in SEQ ID NO:20,

position 550 in intron 5 as defined by the position in SEQ ID NO: 24,

position 37 in intron 15 as defined by the position in SEQ ID NO:37, or

position 345 in intron 18 as defined by the position in SEQ ID NO:40 of the HMG-CoA reductase gene.

According to another aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in HMG-CoA reductase in a human, which method comprises determining the sequence of the nucleic acid of the human at at least one polymorphic position and determining the status of the human by reference to polymorphism in the HMG-CoA reductase gene. Preferred polymorphic positions are one or more of the following positions:

position 1962 in the coding sequence of the HMG-CoA reductase gene as defined by the position in SEQ ID NO: 44, and/or

positions 46 or 267 in the promoter sequence of the HMG-CoA reductase gene as defined by the positions in SEQ ID NO: 45; and/or

position 129 in intron 2 as defined by the position in SEQ ID NO:20,

position 550 in intron 5 as defined by the position in SEQ ID NO: 24,

position 558 in intron 14 as defined by the position in SEQ ID NO:36, or

position 345 in intron 18 as defined by the position in SEQ ID NO:40 of the HMG-CoA reductase gene.

The term human includes both a human having or suspected of having a HMG-CoA reductase mediated disease and an asymptomatic human who may be tested for predisposition

or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

The term single nucleotide polymorphism includes single nucleotide substitution, nucleotide insertion and nucleotide deletion which in the case of insertion and deletion includes insertion or deletion of one or more nucleotides at a position of a gene.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1962 of the coding sequence is presence of A and/or G.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 46 of the promoter is presence of T and/or C.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 267 of the promoter is presence of C and/or G.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 129 of intron 2 is the presence or absence of an insertion of AA.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 550 of intron 5 is presence of T and/or A.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 37 of intron 15 is presence of A and/or G.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 345 of intron 18 is presence of T and/or C.

The method for diagnosis is preferably one in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

Allelic variation at position 1962 consists of a single base substitution from A (the published base), preferably to G.

Allelic variation at position 46 consists of a single base substitution from C (the published case), preferably to G.

Allelic variation at position 267 consists of a single base substitution from T (the published base), preferably to C.

Allelic variation at position 129 consists of a presence or absence of insertion, preferably to presence or absence of the insertion of AA.

Allelic variation at position 550 consists of a single base substitution from T, preferably to A.

Allelic variation at position 37 consists of a single base substitution from A, preferably to G.

Allelic variation at position 345 consists of a single base substitution from T, preferably to C.

The status of the individual may be determined by reference to allelic variation at any one, two, three, four, five, six or seven or more positions.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the PCR. These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
bp	base pair
CMC	Chemical mismatch cleavage
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
HDL	high density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
LCR	Ligase chain reaction
LDL	low density lipoprotein
MASDA	Multiple allele specific diagnostic assay
NASBA	Nucleic acid sequence based amplification
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis

Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™, ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Preferred mutation detection techniques include ARMS™, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, and restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMS™ and RFLP based methods. ARMS™ is an especially preferred method.

In a further aspect, the diagnostic methods of the invention are used to assess the pharmacogenetics of therapeutic compounds in the treatment of HMG-CoA reductase mediated diseases.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the HMG-CoA reductase gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in protein regulation arising as a result of allelic variation may have a direct effect on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by HMG-CoA reductase. This may be particularly relevant in the development of hyperlipoproteinemia and cardiovascular disease and the present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the HMG-CoA reductase gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes.

According to another aspect of the present invention there is provided a human HMG-CoA reductase gene or its complementary strand comprising a polymorphism, preferably corresponding with one or more of positions defined herein or a fragment thereof of at least 20 bases comprising at least one polymorphism.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

According to another aspect of the present invention there is provided a polynucleotide comprising at least 20 bases of the human HMG-CoA reductase gene and comprising a polymorphism selected from any one of the following:

Region	SEQ ID	Position	Polymorphism
Exon 15	SEQ ID NO: 44	1962	A → G
promoter	SEQ ID NO: 45	46	C → G
promoter	SEQ ID NO: 45	267	T → C
Intron 2	SEQ ID NO: 20	129	CT → CAAT
Intron 5	SEQ ID NO: 24	550	T → A
Intron 15	SEQ ID NO: 37	37	A → G
Intron 18	SEQ ID NO: 40	345	T → C

In another embodiment the following polymorphisms are preferred:

Region	SEQ ID	Position	Polymorphism
promoter	SEQ ID NO: 45	46	C → G
promoter	SEQ ID NO: 45	267	T → C
Intron 2	SEQ ID NO: 20	129	CT → CAAT
Intron 5	SEQ ID NO: 24	550	T → A
Intron 15	SEQ ID NO: 37	37	A → G
Intron 18	SEQ ID NO: 40	345	T → C

According to another aspect of the present invention there is provided a human HMG-CoA reductase gene or its complementary strand comprising a polymorphism, preferably corresponding with one or more the positions defined herein or a fragment thereof of at least 20 bases comprising at least one polymorphism.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

The invention further provides a nucleotide primer which can detect a polymorphism of the invention.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a HMG-CoA reductase gene polymorphism, preferably at one or more of the positions as defined herein.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology

Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a HMG-CoA reductase gene polymorphism, preferably at one or more of the positions defined herein.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection.

According to another aspect of the present invention there is provided an allele specific primer or an allele specific oligonucleotide probe capable of detecting a HMG-CoA reductase gene polymorphism at one of the positions defined herein.

According to another aspect of the present invention there is provided a diagnostic kit comprising an allele specific oligonucleotide probe of the invention and/or an allele-specific primer of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase.

In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphisms of relatively high frequency in introns 5 and 18 (see below). The HMG-CoA reductase gene has been mapped to chromosome 5q13.3-q14 (Luskey K.L., Stevens B.;RT "Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. Conserved domains responsible for catalytic activity and sterol-regulated degradation"; J. Biol. Chem. **260**:10271-10277 (1985)). Low frequency polymorphisms may be particularly useful for haplotyping as described below. A haplotype is a set of alleles found at linked polymorphic sites (such as

within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as 2^n haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) **62**:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) **93**:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

According to another aspect of the present invention there is provided a polynucleotide sequence comprising any one of the intron sequences of HMG-CoA reductase defined in any one of SEQ ID NOS: 18-41 herein, an allelic variant thereof, a complementary strand thereof or a fragment thereof. A fragment is at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases. Preferably the allelic variant is one of the SNPs described herein.

According to another aspect of the invention there is provided a polynucleotide sequence comprising any one of the intron sequences of HMG-CoA reductase defined in any one of SEQ ID NOS: 18-41and 54 or a complementary strand thereof or a sequence at least 90% homologous thereto.

The degree of homology may be any of the following: at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology. Homology is determined as follows. "Homology" is a measure of the identity of nucleotide sequences or amino acid sequences. In order to characterize the homology, subject sequences are aligned so that the highest order homology (match) is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. Computer program methods to determine identity between two

sequences, for example, include DNAStar software (DNAStar Inc., Madison, WI); the GCG program package (Devereux, J., et al., Nucleic Acids Research (1984) 12(1):387); BLASTP, BLASTN, FASTA (Atschul, S. F. et al., J Molec Biol (1990) 215:403). Homology (identity) as defined herein is determined conventionally using the well known computer program, BESTFIT (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 53711). When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, about 80% homologous to a reference sequence, according to the present invention, the parameters are set such that the percentage of identity is calculated over the full length of the reference nucleotide sequence or amino acid sequence and that gaps in homology of up to about 20% of the total number of nucleotides in the reference sequence are allowed. Eighty percent of homology is therefore determined, for example, using the BESTFIT program with parameters set such that the percentage of identity is calculated over the full length of the reference sequence and gaps of up to 20% of the total number of amino acids in the reference sequence are allowed, and wherein up to 20% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 20% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. When comparing two sequences, the reference sequence is generally the shorter of the two sequences. This means that for example, if a sequence of 50 nucleotides in length with precise complementarity to a 50 nucleotide region within a 100 nucleotide polypeptide is compared there is 100% identity/homology as opposed to only 50% identity/homology. Percent homologies are likewise determined, for example, to identify preferred species, within the scope of the claims appended hereto, which reside within the range of about 80 percent to 100 percent homology.

According to another aspect of the invention there is provided a polynucleotide sequence comprising any one of the intron sequences of HMG-CoA reductase defined in any one of SEQ ID NOS: 18-41and 54 or a complementary strand thereof or a sequence that hybridises thereto under stringent conditions. As used herein, stringent conditions are those conditions which enable sequences that possess at least 80%, preferably at least 90% and more preferably at least 95% sequence homology to hybridise together. Thus, nucleic acids which can hybridise to the nucleic acid of SEQ ID No. 18-41 or 54 , or the complementary strand thereof, include nucleic acids which have at least 80%, preferably at least 90%, more

preferably at least 95%, still more preferably at least 98% sequence homology and most preferably 100% homology. An example of a suitable hybridisation solution when a nucleic acid is immobilised on a nylon membrane and the probe nucleic acid is greater than 500 bases or base pairs is: 6 x SSC (saline sodium citrate), 0.5% SDS (sodium dodecyl sulphate), 100mg/ml denatured, sonicated salmon sperm DNA. The hybridisation being performed at 68°C for at least 1 hour and the filters then washed at 68°C in 1 x SSC, or for higher stringency, 0.1 x SSC/0.1% SDS. An example of a suitable hybridisation solution when a nucleic acid is immobilised on a nylon membrane and the probe is an oligonucleotide of between 12 and 50 bases is: 3M trimethylammonium chloride (TMACl), 0.01M sodium phosphate (pH 6.8), 1mM EDTA (pH 7.6) , 0.5% SDS,100mg/ml denatured, sonicated salmon sperm DNA and 0.1 dried skimmed milk. The optimal hybridisation temperature (Tm) is usually chosen to be 5°C below the Ti of the hybrid chain. Ti is the irreversible melting temperature of the hybrid formed between the probe and its target. If there are any mismatches between the probe and the target, the Tm will be lower. As a general guide, the recommended hybridisation temperature for 17-mers in 3M TMACl is 48-50°C; for 19-mers,it is 55-57°C; and for 20-mers, it is 58-66°C.

Novel sequence disclosed herein, may be used in another embodiment of the invention to regulate expression of the gene in cells by the use of anti-sense constructs. To enable methods of down-regulating expression of the gene of the present invention in mammalian cells, an example antisense expression construct can be readily constructed for instance using the pREP10 vector (Invitrogen Corporation). Transcripts are expected to inhibit translation of the gene in cells transfected with this type construct. Antisense transcripts are effective for inhibiting translation of the native gene transcript, and capable of inducing the effects (e.g., regulation of tissue physiology) herein described. Oligonucleotides which are complementary to and hybridizable with any portion of novel gene mRNA disclosed herein are contemplated for therapeutic use. Suitable antisense targets include novel intron/ exon junctions disclosed herein. U.S. Patent No. 5,639,595, *Identification of Novel Drugs and Reagents*, issued Jun. 17, 1997, wherein methods of identifying oligonucleotide sequences that display *in vivo* activity are thoroughly described, is herein incorporated by reference. Expression vectors containing random oligonucleotide sequences derived from previously known polynucleotides are transformed into cells. The cells are then assayed for a phenotype resulting from the desired activity of the oligonucleotide. Once cells with the desired phenotype have been identified,

the sequence of the oligonucleotide having the desired activity can be identified. Identification may be accomplished by recovering the vector or by polymerase chain reaction (PCR) amplification and sequencing the region containing the inserted nucleic acid material.

Antisense nucleotide molecules can be synthesized for antisense therapy. These antisense molecules may be DNA, stable derivatives of DNA such as phosphorothioates or methylphosphonates, RNA, stable derivatives of RNA such as 2'-O-alkylRNA, or other oligonucleotide mimetics. U.S. Patent No. 5,652,355, *Hybrid Oligonucleotide Phosphorothioates*, issued July 29, 1997, and U.S. Patent No. 5,652,356, *Inverted Chimeric and Hybrid Oligonucleotides*, issued July 29, 1997, which describe the synthesis and effect of physiologically-stable antisense molecules, are incorporated by reference. Antisense molecules may be introduced into cells by microinjection, liposome encapsulation or by expression from vectors harboring the antisense sequence.

According to another aspect of the present invention there is provided a computer readable medium comprising at least one novel sequence of the invention stored on the medium. The computer readable medium may be used, for example, in homology searching, mapping, haplotyping, genotyping or pharmacogenetic analysis.

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a HMG-CoA reductase inhibitor drug in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in HMG-CoA reductase gene in the human, which diagnosis preferably comprises determining the sequence of the nucleic acid at one or more of the following positions:

position 1962 in the coding sequence of the HMG-CoA reductase gene as defined by the position in SEQ ID NO: 44, and/or

positions 46 or 267 in the promoter sequence of the HMG-CoA reductase gene as defined by the positions in SEQ ID NO: 45; and/or

position 129 in intron 2 as defined by the position in SEQ ID NO:20,

position 550 in intron 5 as defined by the position in SEQ ID NO: 24,

position 37 in intron 15 as defined by the position in SEQ ID NO:37, or

position 345 in intron 18 as defined by the position in SEQ ID NO:40 of the HMG-CoA reductase gene.

and determining the status of the human by reference to polymorphism in the HMG-CoA reductase gene; and

ii) administering an effective amount of a HMG-CoA reductase inhibitor.

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antagonist drug or drugs to administer and/or in deciding on the effective amount of the drug or drugs. Statins already approved for use in humans include atorvastatin, cerivastatin, fluvastatin, pravastatin and simvastatin. The reader is referred to the following references for further information on HMG-CoA reductase inhibitors: Drugs and Therapy Perspectives (12th May 1997), **9**: 1-6; Chong (1997) Pharmacotherapy **17**: 1157-1177; Kellick (1997) Formulary **32**: 352; Kathawala (1991) Medicinal Research Reviews, **11**: 121-146; Jahng (1995) Drugs of the Future **20**: 387-404, and Current Opinion in Lipidology, (1997), **8**, 362 - 368. Another statin drug of note is compound 3a (S-4522) in Watanabe (1997) Bioorganic and Medicinal Chemistry **5**: 437-444.

According to another aspect of the present invention there is provided use of a HMG-CoA reductase antagonist drug in preparation of a medicament for treating a HMG-CoA reductase mediated disease in a human diagnosed as having a single nucleotide polymorphism therein, preferably at one or more of the positions defined herein.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising HMG-CoA reductase antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism therein, preferably at one or more of the positions defined herein.

According to another aspect of the present invention there is provided an allelic variant of human HMG-CoA reductase polypeptide having a valine at position 638 or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises the allelic variant at position 638.

Fragments of polypeptide are at least 10 amino acids, more preferably at least 15 amino acids, more preferably at least 20 amino acids.

According to another aspect of the present invention there is provided an antibody specific for an allelic variant of human HMG-CoA reductase polypeptide having a valine at position 638 or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises the valine at position 638.

Antibodies can be prepared using any suitable method. For example, purified polypeptide may be utilized to prepare specific antibodies. The term "antibodies" is meant to include polycional antibodies, monoclonal antibodies, and the various types of antibody constructs such as for example $F(ab')_2$, Fab and single chain Fv. Antibodies are defined to be specifically binding if they bind the I638V variant of HMG-CoA reductase with a K_a of greater than or equal to about 10^7 M^{-1}. Affinity of binding can be determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice or rats, using procedures that are well-known in the art. In general, antigen is administered to the host animal typically through parenteral injection. The immunogenicity of antigen may be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to antigen. Examples of various assays useful for such determination include those described in: *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radioimmunoprecipitation, enzyme-linked immuno-sorbent assays (ELISA), dot blot assays, and sandwich assays, see U.S. Patent Nos. 4,376,110 and 4,486,530.

Monoclonal antibodies may be readily prepared using well-known procedures, see for example, the procedures described in U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439 and 4,411,993; Monoclonal Antibodies, Hybridomas: *A New Dimension in Biological Analyses,* Plenum Press, Kennett, McKearn, and Bechtol (eds.), (1980).

The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3: 1-9 (1990) which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7: 394 (1989).

Once isolated and purified, the antibodies may be used to detect the presence of antigen in a sample using established assay protocols, see for example "A Practical Guide to ELISA" by D. M. Kemeny, Pergamon Press, Oxford, England.

According to another aspect of the invention there is provided a diagnostic kit comprising an antibody of the invention.

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™ ,available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR. Where described below, the primary fragment was diluted 1/100 and two microlitres were used as template for amplification of secondary fragments. PCR was performed in two stages (primary fragment then secondary fragment) to ensure specific amplification of the desired target sequence.

Single nucleotide polymorphism at position 1962 of SEQ ID NO: 44

This polymorphism was detected by amplification of a primary fragment from genomic DNA, followed by amplification of a secondary fragment, followed by dye primer sequencing with M13F primer:

Primary Fragment

Forward Oligo, SEQ ID NO: 1

Reverse Oligo, SEQ ID NO: 2

Annealing Temp 68°

Time 1 min

Secondary Fragment

Forward Oligo, SEQ ID NO: 3

Reverse Oligo, SEQ ID NO: 4

Annealing Temp 69°

Time 1 min

Single nucleotide polymorphisms at positions 46 and 267 of SEQ ID NO: 45

These polymorphisms were detected by amplification of a primary fragment from genomic DNA, followed by dye terminator sequencing using the same oligos.

Forward Oligo SEQ ID NO: 5

Reverse Oligo SEQ ID NO: 6

Annealing Temp 64°

Time 2 min

Single nucleotide polymorphisms at position 129 of HMG CoA reductase intron 2 sequence (SEQ ID NO: 20)

This polymorphism was detected by amplification of a primary fragment from genomic DNA, followed by dye terminator sequencing.

Primary Fragment

Forward Oligo SEQ ID NO: 7

Reverse Oligo SEQ ID NO: 8

Annealing Temp 53°

Time 1 min

Dye terminator sequencing oligo SEQ ID NO: 9

Single nucleotide polymorphisms at position 550 of HMG CoA reductase intron 5 sequence SEQ ID NO: 24 (T to A).

This polymorphism was detected by amplification of a primary fragment from genomic DNA, followed by dye primer sequencing with M13F primer:

Primary Fragment

Forward Oligo SEQ ID NO: 42

Reverse Oligo SEQ ID NO: 43

Annealing Temp 69°

Time 1 min

Single nucleotide polymorphisms at position 37 of HMG CoA reductase intron 15 sequence SEQ ID NO: 37 (A to G).

This polymorphism was detected by amplification of a primary fragment from genomic DNA, followed by amplification of a secondary fragment, followed by dye primer sequencing with M13F primer:

Primary Fragment

Forward Oligo SEQ ID NO: 10

Reverse Oligo SEQ ID NO: 11

Annealing Temp 68°

Time 1 min

Secondary Fragment

Forward Oligo SEQ ID NO: 12

Reverse Oligo SEQ ID NO: 13

Annealing Temp 69°

Time 1 min

Single nucleotide polymorphisms at position 345 of HMG CoA reductase intron 18 sequence

This polymorphism was detected by amplification of a primary fragment from genomic DNA, followed by dye terminator sequencing.

Primary Fragment

Forward Oligo SEQ ID NO: 14

Reverse Oligo SEQ ID NO: 15

Annealing Temp 58°

Time 1 min

Dye terminator sequencing oligo SEQ ID NO: 16

Dye Primer Sequencing

Dye-primer sequencing using M13 forward and reverse primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with "AmpliTaq FS"‰ DNA polymerase, modified in that the annealing temperature was 45 ° and DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

Dye Terminator Sequencing

Dye-terminator sequencing was as described in the ABI protocol P/N 4303150 for the ABI Prism™ Big Dye terminator cycle sequencing core kit with "AmpliTaq FS" ™ DNA polymerase.

The extension reactions were ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

2. Results

Exon-Intron Organisation of the Human HMG-CoA Reductase Gene

Exon sequences are in capital letters: intron sequences (where shown) are in lowercase letters. The number shown immediately below the DNA sequence denotes the nucleotide position from SEQ ID NO: 44 at which the intron interrupts the HMG CoA reductase mRNA. The 5' boundary and sequence of intron 1 are as described by K.L.Luskey, Mol.Cell.Biol. 7:1881-1893 (1987), Medline ref. No.87257890.

Intron no.	Sequence of Exon-Intron Junctions		Intron size (Kb)
	5' Boundary	3' Boundary	
1.	GAT CTG GAG gtgagg(SEQ ID NO: 17)..ATG TTG TCA 51		4.5 approx
2.	TTT GAG GAG ..... GAT GTT TTG 215 216		1.2 approx
3.	ATA TTT TGG .....GTA TTG CTG 327 328		0.28
4.	AGG CTT GAA .....TGA AGC TTT 415 416		1.222
5.	AAC TCA CAG.....GAT GAA GTA 500 501		1.7 approx
6.	CCA TGT CAG..... GGG TAC GTC 606 607		2 approx
7.	GTA TTA GAG .....CTT TCT CGG 713 714		0.11
8.	ATG ATT ATG..... TCT CTA GGC 830 831		0.414
9.	TCT CTC TAA ..... AAT GAT CAG 991 992		0.12
10.	AAA GAA AAG ..... TTG AGG TTA 1239 1240		0.108
11.	AAT GCA GAG ..... AAA GGT GCA 1418 1419		4 approx
12.	TAC TCC TTG.....GTG ATG GGA 1613 1614		0.358
13.	GCA ATA GGT ..... CTT GGT GGA		0.15

	1772 1773		
14.	CAC TAG CAG .....ATT TGC ACG		1.5 approx
	1930 1931		
15.	ATT TCA AAG ..... GGT ACA GAG		2 approx
	2036 2037		
16.	GTC AGA GAA ..... GTA TTA AAG		0.343
	2207 2208		
17.	TGT GGA CAG ..... GAT GCA GCA		0.088
	2348 2349		
18.	TGT TTG CAG ..... ATG CTA GGT		0.428
	2507 2508		
19.	TCA CAA CAG .....GTC GAA GAT		0.149
	2662 2663		

Polymorphisms

SEQ ID NO: 44

Nucleotide 1962	A /G	Ile/Val (638)	**ATA/GTA**	**ATA**	95.5 %
				GTA	4.5 %

The allele frequencies were based on analysis of 22 individuals. A was the published base. This change in amino acid sequence is within the catalytic domain of the polypeptide and may therefore be of particular interest.

SEQ ID NO: 45

Nucleotide 46	C/G	Allele Frequency	C	95.8 %
			G	4.2 %

C was the published base.

Nucleotide 267	T/C	Allele Frequency	T	95.8 %
			C	4.2 %

T was the published base. These changes in the promoter may affect transcript levels. The allele frequencies were based on analysis of 24 individuals.

HMG CoA Reductase intron 2 sequence

Nucleotide 129 of SEQ ID NO: 20 Insertion of AA

Allele Frequency

CT	95 %

CAAT 5 %

Allele frequencies determined in a panel of 20 individuals

HMG CoA Reductase intron 5 sequence

Nucleotide 570 of SEQ ID NO: 24 T/A

Allele Frequency T 72.7 %

A 27.3 %

The allele frequencies were based on analysis of 22 individuals.

HMG CoA Reductase intron 15 sequence

Nucleotide 37 of SEQ ID NO: 37 A/G

Allele Frequency A 97.7 %

G 2.3 %

The allele frequencies were based on analysis of 22 individuals.

HMG CoA Reductase intron 18 sequence

Nucleotide 345 of SEQ ID NO: 40 T/C

Allele Frequency C 61.7 %

T 28.3 %

The allele frequencies were based on analysis of 23 individuals.

Summary of Polymorphisms

SNP	Ref	Position	Change
Exon 15	SEQ ID NO: 44	1962, 638	A → G, Ile → Val
promoter	SEQ ID NO: 45	46	C → G
promoter	SEQ ID NO: 45	267	T → C
Intron 2	SEQ ID NO: 20	129	CT → CAAT
Intron 5	SEQ ID NO: 24	550	T → A
Intron 15	SEQ ID NO: 37	37	A → G
Intron 18	SEQ ID NO: 40	345	T → C

Intron Sequence

Intron 1 sequence (Last 634bp)

SEQ ID NO: 18

Intron 2 sequence

First 506bp, SEQ ID NO: 19

Last 230bp, SEQ ID NO: 20

Intron 3 sequence (280bp)

SEQ ID NO: 21

Intron 4 sequence (1,222bp)

SEQ ID NO: 22

Intron 5 sequence (First 850bp and last 730bp)

SEQ ID NO: 23

SEQ ID NO: 24

Intron 6 sequence (First 492bp and last 715bp)

SEQ ID NO: 25

SEQ ID NO: 26

Intron 7 sequence (109bp)

SEQ ID NO: 27

Intron 8 sequence (414bp)

SEQ ID NO: 28

Intron 9 sequence (118bp)

SEQ ID NO: 29

Intron 10 sequence (108bp)

SEQ ID NO: 30

Intron 11 sequence (First 728bp and last 291 bp[1])

SEQ ID NO: 31

SEQ ID NO: 54

Intron 12 sequence (358bp)

SEQ ID NO: 33

Intron 13 sequence (150bp)

SEQ ID NO: 34

[1] last 30 bp shown as SEQ ID NO: 32

Intron 14 sequence (First 247bp and last 594bp)

SEQ ID NO: 35

SEQ ID NO: 36

Intron 15 sequence (First 357bp)

SEQ ID NO: 37

Intron 16 sequence (342bp)

SEQ ID NO: 38

Intron 17 sequence (87bp)

SEQ ID NO: 39

Intron 18 (427 bp)

SEQ ID NO: 40

Intron 19 sequence (148bp)

SEQ ID NO: 41

Example 2

Single nucleotide polymorphism at position 915 of HMG CoA reductase intron 4 sequence SEQ ID No: 22 (Deletion of T)

This polymorphism was detected by amplification of a primary fragment of genomic DNA, followed by a secondary fragment, followed by dye terminator sequencing.

a) Primary fragment

Forward oligo SEQ ID No: 49, Reverse oligo SEQ ID No: 47

Annealing temperature 55°C, Time 1 min

b) Secondary fragment

Forward oligo SEQ ID No. 48, Reverse oligo SEQ ID No. 46

Annealing temperature 55°C, Time 1 min

Dye terminator sequencing oligo; SEQ ID No: 50

Example 3

ARMS™ Diagnostic Assay To Detect Exon 15 Polymorphism

ARMS™ assay technology is described in Chapter 11 of the textbook PCR by C R Newton & A Graham, 2nd Edition, BIOS Scientific Publishers Ltd, Oxford, UK. Below are the primer sequences needed to carry out a diagnostic ARMS™ assay on the exon 15 polymorphism, in order to detect which allele is present.

The following primers amplify a 198 base pair PCR product only if the A allele is present:

Constant primer (forward): SEQ ID NO: 51

A allele specific primer (reverse): SEQ ID NO: 52

Annealing temp. 68°C, Time 45secs

The following primers amplify a 198 base pair PCR product only if the G allele is present:

Constant primer (forward): SEQ ID NO: 51

G allele specific primer (reverse): SEQ ID NO: 53

Annealing temp. 68°C, Time 45secs

Sequence listing free text

For SEQ ID NO: 46-49 & 51-53:

<223> Description of Artificial Sequence:PCR primer

For SEQ ID NO: 50:

<223> Description of Artificial Sequence:dye terminator sequencing oligo

CLAIMS

1. A method for the diagnosis of a single nucleotide polymorphism in HMG-CoA reductase in a human, which method comprises determining the sequence of the nucleic acid of the human at at least one polymorphic position selected from one or more of the following positions:

position 1962 in the coding sequence of the HMG-CoA reductase gene as defined by the position in SEQ ID NO: 44, and/or

positions 46 or 267 in the promoter sequence of the HMG-CoA reductase gene as defined by the positions in SEQ ID NO: 45; and/or

position 129 in intron 2 as defined by the position in SEQ ID NO:20, and/or

position 550 in intron 5 as defined by the position in SEQ ID NO: 24, and/or

position 37 in intron 15 as defined by the position in SEQ ID NO:37, and/or

position 345 in intron 18 as defined by the position in SEQ ID NO:40 of the HMG-CoA reductase gene, and

determining the status of the human by reference to polymorphism in the HMG-CoA reductase gene.

2. A method according to claim 1 in which the polymorphism is further defined as the following:

the single nucleotide polymorphism at position 1962 of the coding sequence is presence of A and/or G;

the single nucleotide polymorphism at position 46 of the promoter is presence of T and/or C.

the single nucleotide polymorphism at position 267 of the promoter is presence of C and/or G;

the single nucleotide polymorphism at position 129 of intron 2 is the presence or absence of an insertion of AA;

the single nucleotide polymorphism at position 550 of intron 5 is presence of T and/or A;

the single nucleotide polymorphism at position 37 of intron 15 is presence of A and/or G; and

the single nucleotide polymorphism at position 345 of intron 18 is presence of T and/or C.

3. A method according to claim 1 comprising determining the sequence of the nucleic acid of the human at position 1962 in the coding sequence of the HMG-CoA reductase gene as defined by the position in SEQ ID NO: 44 for presence of A and/or G.

4. A method according to any preceding claim in which the sequence is determined by a method selected from amplification refractory mutation system and restriction fragment length polymorphism.

5. Use of a method as defined in any preceding claim to assess the pharmacogenetics of therapeutic compounds in the treatment of HMG-CoA reductase mediated diseases.

6. A polynucleotide comprising at least 20 bases of the human HMG-CoA reductase gene and comprising a polymorphism selected from any one of the following:

Region	SEQ ID	Position	Polymorphism
Exon 15	SEQ ID NO: 44	1962	A → G
promoter	SEQ ID NO: 45	46	C → G
promoter	SEQ ID NO: 45	267	T → C
Intron 2	SEQ ID NO: 20	129	CT → CAAT
Intron 5	SEQ ID NO: 24	550	T → A
Intron 15	SEQ ID NO: 37	37	A → G
Intron 18	SEQ ID NO: 40	345	T → C

7. An allele specific primer or an allele specific oligonucleotide probe capable of detecting a HMG-CoA reductase gene polymorphism at one of the positions as defined in the table of claim 6.

8. Use of any polymorphism as defined in the table of claim 6 as a genetic marker in linkage studies.

9 A computer readable medium comprising at least one polymorphism as defined in the table of claim 6 stored on the medium.

10 Use of a HMG-CoA reductase antagonist drug in preparation of a medicament for treating a HMG-CoA reductase mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of the positions defined in the table of claim 6.

11 An allelic variant of human HMG-CoA reductase polypeptide comprising a valine at position 638 or a fragment thereof comprising at least 10 amino acids provided that the fragment comprises the valine at position 638.

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ttttaacaaa tattttgagc aactactatg tttagctttg aggatgccaa agaaatatag 1080
ggtatacttt tttgtctgca gaaaggtaca catactacag gatcatacag tatggagggg 1140
gaaaggtttt gttctaaaaa gaatttttt aaaatcatac tttttcccgt taaatttatc 1200
tgatcatttt gttctttttcc ag 1222

<210> 23
<211> 849
<212> DNA
<213> Homo sapiens

<400> 23
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aattctcaag cccaagactg gtctccataa gtgccccagg aacagtcccc tgtatctaac 180
aactcaatta tgattctgta gctactggaa tttggaattn cccccatttt tctttttgaa 240
agttttcaga acttntggtn ataataattt ttgggtnaat aagagtattt tcctagtgaa 300
acacatcaga gagcagaaca agatctaatg gaagagaaac ccagggtaga ttgattgatt 360
gattgatttg agatgtcgtc tcacnctnct acccngngct gnnttgctcn nggtnagatc 420
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tgttcncatg ctctncncnn tattcttttng ccantgnttg gctcaagact cgccccttca 600
tnttctttgc accntcaann cgnacccncc cgcnctcccc cttccctagt gcgcgngnat 660
gttcngcttn gancccnctnc tncctgngcc atntttnntc ttanaccccn naaatncacn 720
cttggccctt tntnntccta ttnttacnct tcnntctttn acgtgncact ntntancttt 780
tnacnnaccc tnancctctt tnccacntnt cctnttnnct atnnctctgc ctctttnannt 840

ncccacct 849

<210> 24
<211> 730
<212> DNA
<213> Homo sapiens

<400> 24
cgaaaaangc cccattcncc cnatattggn aagaaggggg atcnttgaca tatacaagga 60
ataggtgggt ttatgaatcn aatangtctc ataaatttcn aactaagctt ctgtcaggta 120
ggtaaaatat agaangtgna ggatttaatt aggggattaa atgccagagt aatnccctnc 180
ctcaaaggaa tantcctacc aaatatcnna ttcagggaaa ggaatcaggg cnctatatgt 240
tctttttaa aattgggcng ggcccagggc tcncacctga attccagcac ttcggnagnc 300
cgaggtgggc agatcncctg aggcaggagc tccagaccag cctggccaac ctggtgaaac 360
ccagtctcta ctaaaaatac aaaaattagc tgggcatggt ggcgggtgcc tgtaatccca 420
gctactcggg aggctgaggc aggagaattg cttgaaccca ggaggcagag gtngcagtga 480
accaagatca caccattgca cattgcactc cagcctggga aacaaagtga gactacatct 540
caaaaaaaaa ttttttaaa tcctttatat tacaatcata ctttgtatct tgaatacctg 600
ttagttttat catattgtat attttactct ttgaatagta atttgatatt aatataagcc 660
ataggatgct ctaacattta aaaaagttgt tctgtccctt gccttcattg atatgtttga 720
tctgttttag 730

<210> 25
<211> 491
<212> DNA
<213> Homo sapiens

<400> 25
gtttgtaagc aatttttgcc atattttaaa ataggtatgt cgctaaagag gaaaaagaac 60
atcttggatt tgtattattt attgttanat tctgactttt aaattactct taaaattttt 120
tattatatta gtgtgtgggt atatctggcc tgtttgcttt ggtggaaact tagcagcagg 180
ttactgattt atttttcact cctgccanct actttgtgng catnactntg tgatattttt 240
attcattnga tntctttngt tnggtttttt anccaacatt gcattccaag gttggtctgg 300
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nttaattntc atgtagggac gaaaagagtc aggaattggg accagatctg gagtaaggat 420
tcacctnata aggnggattg ggaantattt aaatccggat aagtaagccg gaccatcttc 480
agcaagtacc c 491

<210> 26
<211> 715
<212> DNA
<213> Homo sapiens

<400> 26
cgttttctat tttgagatcc cccaaatgaa tccctttaac aagtgtgaag tacaatatgt 60
ttggttaatt tttagtctta agttgggcat tttgtattat atcattttcc aacagatcaa 120
tgaaataacc aaaattataa gaacttcagt aggcatcata gaggttatat tgaaaattag 180
agtttgttgg tacacaaaaa atattatttt gaccttatat caggactggc ataactggca 240
ggatattcta cttatataaa aaatccttgg ttaattggca aattgctttt ctcctaacaa 300
gtgggattag atcaatagtg tcactggggt tttgctctgt tagagtagcc tgtcctctcc 360
ttatttaata ataaggcact actgcttgac aaaaaggaat tggaaacaca tatgttttat 420
caattgtgta ttaaacacta ccattctgcc tggcattgtg atatggggac atgagagaag 480
gcaagagctt tgctcttgag ggacttagag ttctgttgtt attcctgctg tttcctaagg 540
cttggcatca cctctaagtt gctaattcta tttccagtaa gtggcaagga gcttaatgta 600
ctaatatttt catgttttgt ccacctgcag gaagacaaat atatccttgt gatatatgca 660
gcataaaaaa taacgtagac tttactagtt gtatctttaa tttttctcta accag 715

<210> 27
<211> 109
<212> DNA
<213> Homo sapiens

<400> 27
gtaagcccaa ttcttacata tggcactagt agaagagtaa gatttctgct tacacagttt 60
acctaaacag aatcaatacc ttctaatgtc acactgactt aatttgtag 109

<210> 28
<211> 414
<212> DNA
<213> Homo sapiens

<400> 28
gtaatgacat ggttttcttc ttcttttagt atcctcagtt ccaatctcat tatttttaag 60
atttcttttt ttctacaatt ttggcccatt caatgatatt gcacccccct cttccttttt 120
ttcttaatgt gttcatttct ttgaggctcc tggtctttat tagcccctct ctcctaaaca 180
gacttttttaa gttcccccac cttatctctc gttgaaagcc tgttctttgg ggtgttttca 240
gtagttcagt ggggtcacta ctttagttag ttgcatagca agcttggggc ttttttttt 300
tcatggtaag gggaagctat gagagataat gtctggctgt ccagttgcta gggataagaa 360
atttaagttc tattgatatg cagaggatac attactttaa aaattttatt tcag 414

<210> 29
<211> 118
<212> DNA
<213> Homo sapiens

<400> 29
gtaagttaat tgaaatctac tttgtgatat attaatcata acactctatg ctaatgtaag 60
tttagattgt gtcctttaca tttctgaata agattttaat ttgctttctt ttatttag 118

<210> 30
<211> 108
<212> DNA
<213> Homo sapiens

<400> 30
gtaacttgtt attctcttcg ctttcaatcc ttcattgctt tgtcaaaaag tagtctgttt 60
tcaaaattat gtgccgtgtt gtgagatttc ttttgatttc ttgaacag 108

<210> 31
<211> 728
<212> DNA
<213> Homo sapiens

<400> 31
gtgaggatga taacataaac tccaatgtgg cattttttcat tacaaaggng cttngnnaag 60
gangaaaaat ctagtatctg ctgaacactn cagctaagtt ctgggcacgg tgtancatga 120
ctaacagata ctatcttctt tctttatttc acacaacctt gagaggtagg tncaattatc 180
tatttttcag atgagaacat tgaggctcca atatgtttaa tttcccaaag nagtccctct 240
nggaaatgat naagctgata gnagggtcca agatttttctg actccagagt caaaactctt 300
tctagtttat tactgcttat catagagatg agtgactact gtatnctcat aggngtgntg 360
aggcctagaa agagtttacc acagagacaa gtttcaaaga tagangaaag tttgttttng 420
tnttgtttng nngctggata cccatgagga agtttgcttt tctttctgac atttgaacag 480
gaccttntgc ctacatgacc atatgaatct acttatgctt tcatgcaaan aatcatggtt 540
ccatncatgt ctgcttnaca cgggtgtttc ttttaannca caggntaatt ncgtttaatt 600
gggnaaaatg ccatttttttg gccagccttt tttgagggtt tcttggccaa antttttttt 660
gnatantnnt gatnnataat gattattatc nctngntttg gagacaaaan ntnnctttttt 720
tcccccag 728

<210> 32
<211> 30
<212> DNA
<213> Homo sapiens

<400> 32
caatttcatt ttttttctcc atttctttag 30

<210> 33
<211> 358
<212> DNA
<213> Homo sapiens

<400> 33
gtatgttatt ttctcgatta agagagattt gctttgtatg tttttaatct ttttttcttga 60
ttagtttcat atatgtacat agttttataa aacattttcc ttttaaatca ttttatccta 120
atttttttatt ctgcttatga tgtaggtcat agaaattaaa aatatatttc ctgcttttat 180
agtcattact caaagatttt agtattttaa acacttttta aaggtgaatt aaacattttg 240
tttaaaaaga atacatacta aaggattaag tttgaagata gttatactga caagctgaga 300
taaaattttg tgcatttact atatagattt tcatttggtg cctgacttta ccttttag 358

<210> 34
<211> 150
<212> DNA
<213> Homo sapiens

<400> 34
gtaagttggc atttatatat ttgccagttt aaaaatacat cataagtaag gcaatgagaa 60
gagttttaag gacaattagt gataccttt gggtcaagca tgagcatttt tgggtaacat 120
gtgcttgctt ctctaacata tactgtgtag 150

<210> 35
<211> 246
<212> DNA
<213> Homo sapiens

<400> 35
gtgtgtgagt ggatttgtat gtacagttat atctatttgt ttattttaga accagtgtca 60
ttttctgtga ttaccaaaca taattgttaa catattacct gctaangagc acataacaga 120
atatcaactt taaagccatt cattnaaaat gagtaatatt tatgctgggn ngggggggaaa 180
aaaagaatgt ngatncaaat gaatngctcc ncagaggtaa attagtaaga aaaaaaaaaa 240
gggggg 246

<210> 36
<211> 594
<212> DNA
<213> Homo sapiens

<400> 36
aaaaaaaaaa aaaaagtgta anctagtaat ttttgattag atgttacttt gcctaggana 60
gaactgtttt agaaaaaaag atttttcaaa taggagagaa atattagtat aataagactt 120
ccttcaaata aagaaaatta ataaagtagc ataatcaacn caaatgatan ccatagtata 180
gttcaagctt aacacatttg tttttatgtg aactgtgtaa gtttattaag aaataattgt 240
gactgggcgc agtggctcac gcctgtaatc ccaacacttt ggggaggcca acgcgggcag 300
atcacttgag gccaggagtt cgagaccagc ctggccanca tggcgaaacc ctgtctctac 360
tcaaaataca aaaattggct gggcatggtg gcccgcgcct gtaatcccag ctactcggga 420
ggctgaggct ggagaatttc ttgaacccgg gaggtggatg ttgcagtgag ccaagatcaa 480
gccactgcac tccagcctgg gccacagagt gagactccgt ctcaaaaaan aacaaaaaac 540
aaagaaataa taataataaa agaataaaac acagtctttg catctttntt atag 594

<210> 37
<211> 357
<212> DNA
<213> Homo sapiens

<400> 37
gtaagnntng ccagantntn tnaangtcct tttattaant ntttnnnctt ttataaaaaa 60
caaatcagcc cttttgttga tggncattcn ttncnttnga nngattcant ttanantngg 120
cnttacacat atcctgggtt tacttaggac gggnaacant nttagtntng acatttcaaa 180
actttntcca gtcaananac cncntttgag gctgacctct ncaagattng tntttaanan 240
cnccantatn ttttcngcct tnggnaggcc nnggcaagaa gnttgnttgg ggtntgaagn 300
tnaanaccag ccngggcnac acananagat gctntntcta naaacaataa aaaaaaa 357

<210> 38
<211> 342
<212> DNA
<213> Homo sapiens

<400> 38
gtgagtgact ggatggataa tttatctttt ttattttgna atctttaatt gtatttaaaa 60
atgggggaaa ggagtattaa cattttaaat aaagttaaat atatgggaca gtgttttcca 120
tcaaagatga ctgttgtacc ttgcccatct gtctgtgtgn atcatccata ggaacaaact 180
ttactgattt tttttaattt tttttatttt ttaatggagg acagggctta aatggggcca 240
catctaaact ttgttttctg gaggttcaga aagatagatt tgggtaacat tcccctgaac 300
cttctggagg aacatctaaa tgtacacagc tctgttttgt ag 342

<210> 39
<211> 87
<212> DNA
<213> Homo sapiens

<400> 39
gtgagctctc cagcctccac ttctcttgtg ttacgtcttt ctaagtgaaa gaagtatatg 60
gtatattttt tcttttcttg tttccag 87

<210> 40
<211> 428
<212> DNA
<213> Homo sapiens

<400> 40
gtatgatgta tcaggcatag agtccacaag cctagttctg actctctggg tttctctttc 60
tatctgagac tatgtatcac tcacctctat tttaattggt cttttccaaa ctcttttgtc 120
atatcagcct aatccattgt gtccaaataa gcatgtttaa gcttatgctt agataagaaa 180
gtagatgaag agagcaaatg aatgttcatc tactgagtta agggtactgc cagtcaggct 240
gtgaatatta tgttagctat ggtattatgc actgtcaggt gtggctgtca agtcttggaa 300
agttagtgct tccagtggag tctagttcta ttctgatgcc attatagttg ccctgttttt 360
agttgattta gtaagaaatt ggtcatgatt ttaagggtga atcttgttgt gtctctccct 420
ggctacag 428

<210> 41
<211> 148
<212> DNA
<213> Homo sapiens

<400> 41
gtaagactca aagatatatt taacatgttc cccctatact tcaaaaaata tgcagtgtaa 60
aaacttacta ttcatctact gtagttccaa gttaaaattc tacactcctg atatttatat 120
attgctactt tgtcattttc taccatag 148

<210> 42
<211> 42
<212> DNA
<213> Homo sapiens

<400> 42
aacgacggcc agttcaggag ctccagccag ctggcaacct gg 42

<210> 43
<211> 31
<212> DNA
<213> Homo sapiens

<400> 43
ggcaggagtg aaaaataaat cagtaacctg c 31

<210> 44
<211> 2904
<212> DNA
<213> Homo sapiens

<400> 44
ttcggtggcc tctagtgaga tctggaggat ccaaggattc tgtagctaca atgttgtcaa 60
gacttttttcg aatgcatggc ctctttgtgg cctcccatcc ctgggaagtc atagtgggga 120
cagtgacact gaccatctgc atgatgtcca tgaacatgtt tactggtaac aataagatct 180
gtggttggaa ttatgaatgt ccaaagtttg aagaggatgt tttgagcagt gacattataa 240
ttctgacaat aacacgatgc atagccatcc tgtatattta cttccagttc cagaatttac 300
gtcaacttgg atcaaaatat attttgggta ttgctggcct tttcacaatt ttctcaagtt 360
ttgtattcag tacagttgtc attcacttct tagacaaaga attgacaggc ttgaatgaag 420
ctttgcccct tttcctactt ttgattgacc tttccagagc aagcacatta gcaaagtttg 480
ccctcagttc caactcacag gatgaagtaa gggaaaatat tgctcgtgga atggcaattt 540
taggtcctac gtttaccctc gatgctcttg ttgaatgtct tgtgattgga gttggtacca 600
tgtcaggggt acgtcagctt gaaattatgt gctgctttgg ctgcatgtca gttcttgcca 660
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aagaagaaaa taagccgaat cctgtaactc agagggtcaa gatgattatg tctctaggct 840
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cagatacttc taaggtttca ttaggactgg atgaaaatgt gtccaagaga attgaaccaa 960
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cagtagagga agagacaggg ataaaccgag aaagaaaagt tgaggttata aaacccttag 1260
tggctgaaac agatacccca aacagagcta catttgtggt tggtaactcc tccttactcg 1320
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ctaatgaaga atgtctacag atacttggga atgcagagaa aggtgcaaaa ttccttagtg 1440
atgctgagat catccagtta gtcaatgcta agcatatccc agcctacaag ttggaaactc 1500
tgatggaaac tcatgagcgt ggtgtatcta ttcgccgaca gttactttcc aagaagcttt 1560
cagaaccttc ttctctccag tacctacctt acagggatta taattactcc ttggtgatgg 1620
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gtggtaacta ttgtactgac aagaaacctg ctgctataaa ttggatagag ggaagaggaa 2160
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ccaagaagac agcctgaata gcccgacagt tctgaactgg aacatgggca ttgggttcta 2760
aaggactaac ataaaatctg tgaattaaaa aagctcaatg cattgtcttg tggaggatga 2820
ataaatgtga tcactgagac agccacttgg tttttggctc tttcagagag gtctcaggtt 2880
ctttccatgc agactcctca gatc 2904

<210> 45
<211> 1227
<212> DNA
<213> Homo sapiens

<400> 45
tggtccccta tcgcctccgc ctagcagctg ccatcggtgc gcccccacag ctctaggacc 60
aataggcagg ccctagtgct gggactcgaa cggctattgg ttggccgagc cgtggtgaga 120
gatggtgcgg tgcctgttct tggccctgca gagagctgtg ggcggttgtt aaggcgaccg 180
ttcgtgacgt agcgccgtca ggccgagcag cccccaggcg attggctaga caatcgaacg 240
atcctctctt attggtcgaa ggctcgtcca gctccgagcg tgcgtaaggt gagggctcct 300
tccgctccgc gactgcgtta actggagcca ggctgagcgt cggcgccggg gttcggtggc 360
ctctagtgag atctggaggt gaggcgggcg gtgaccgaga agaggggcag gggcggcggg 420
gagcggggcg agatgggtgg gagcggggtt tgggctgtgt tggtggcaat tctggagctt 480
ccctcggccc tgggaagtgg ctaccggcag ctcctgcgga cctggagggg gctgcggttg 540
cgctttgtcg gtgtggcagc tcggacccgc ggggactgca aggaatgtcc ttgaggcccg 600
gcaggccgag cggcggccgg catcagtgcc ggagtaaccc ggggtcccgg ggtgggcttg 660
agaggcgggc ggcggtctgg cctcttcgtg actgcggtca tcatcggtgg acccgcgggg 720

cgtagctgcg ttcatcgtcc ctgttcagtc agagtaggca gtgctggctg cacggtcacg 780
aaaatcgggg cggaaagggt gtcaggcagg gtgacctcgg aggcccctgg attcgagaaa 840
tgctaggggt ctatggggct gtcgggccgg cagctcgcag ggcagacggg agaagcgcct 900
gcatcccggg atccggcatt ctcgccagga actgctgttc gttagcacct ttcttttagg 960
tgacgggaaa gatctctgta aatactgctg actaacttag aaccatgaaa gaaccgtgga 1020
ttggtgtaga tgtgtctggt tatttacagg agaacggctt gagaggatgc ggagcccaac 1080
gtgggacttc gcacaatgac tcaaaagatt cttctccctc ttttttttct ttttttttg 1140
gtaaggggtg tagtctcctt ggtgctgata ttcttttagg aaaaatgtac cttggagata 1200
caaatataga acagttaatt tctgcag 1227

<210> 46
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 46
tgtaaaacga cggccagtag gaatactatt cacattccta tc 42

<210> 47
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 47
tgtaaaacga cggccagtct ttgggcaatt cttaaatctt gtgc 44

<210> 48
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 48
gcaaactcct gcctcaagtg atccgcctgc 30

<210> 49
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:PCR primer

<400> 49
gttcaagcag ttctcctgcc tcagcctcct g 31

<210> 50
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:dye terminator sequencing oligo

<400> 50
agaaaggtac acatactaca gg 22

<210> 51
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 51
atgttgcagt gagccaagat caagc 25

<210> 52
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 52
aacggatata aaggttgcgt ccagcagt 28

<210> 53
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR primer

<400> 53
aacggatata aaggttgcgt ccagctcc 28

<210> 54
<211> 291
<212> DNA
<213> Homo sapiens

<400> 54
ttcacattta ttttttcttt tatggtgtat tagtgcaagc ctgtctttgt attgtaaaat 60
ctaatgatac ggtatttata ttatttttgt ttggcatttt ttgcattaaa tgaattattt 120
tgcagaggta tcttttaatt aaaaactaca gtgatttaat ttaaaaatta cattatttta 180
gcttagcatt gtttgtatta aatggtttat aacatgaaat acagtccttc aagtcttctg 240
tttcatctct ctctctgacc acaatttcat tttttttctc catttcttta g 291

INTERNATIONAL SEARCH REPORT

Int. Ional Application No
PCT/GB 00/02396

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N9/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, WPI Data, PAJ, EMBL, MEDLINE, CHEM ABS Data, EMBASE, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Database entry MARED01, acc.nos x00494 , L00165, "Hamster HMG CoA reductase" XP002151037 abstract ---	6,11
X	DATABASE EMBL 'Online! Database entry HSHMGCOA, acc.no. M11058, 2 July 1986 (1986-07-02) "Human 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA" XP002151038 cited in the application	6
Y	abstract --- -/--	1-5,7-11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 October 2000	07/11/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Reuter, U

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Database entry HSHMGCOB, acc.no. M15959, 2 April 1988 (1988-04-02) "Human HMG CoA reductase gene , exon 1 , and promoter region" XP002151039 cited in the application	6
Y	abstract	1-5,7-11
Y	WO 98 45477 A (EURONA MEDICAL AB ;LINDSTROEM PER HARRY RUTGER (SE); ANDERSSON MAR) 15 October 1998 (1998-10-15) the whole document	1-5,7-11
Y	SCHAFER A J ET AL: "DNA VARIATION AND THE FUTURE OF HUMAN GENETICS" NATURE BIOTECHNOLOGY,NATURE PUBLISHING,US, vol. 16, January 1998 (1998-01), pages 33-39, XP000890128 ISSN: 1087-0156 the whole document	1-5,7-11
A	ALROKAYAN S A H: "Polymorphism in the exon 1 of the human HMGCoA reductase gene." FASEB JOURNAL, vol. 11, no. 9, 1997, page A1210 XP002151035 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual Meeting of the American Society for Biochemistry and Molecular Biology;San Francisco, California, USA; August 24-29, 1997 ISSN: 0892-6638 abstract	1-11
A	LEITERSDORF, ERAN ET AL: "ScrFI polymorphism in the 2nd intron of the HMGCR gene" NUCLEIC ACIDS RES. (1990), 18(18), 5584 , XP002151036 the whole document	1-11
A	BERGER, G. M. B. ET AL: "HMG-CoA reductase is not the site of the primary defect in phytosterolemi" J. LIPID RES. (1998), 39(5), 1046-1054 , XP000952754 the whole document	1-11

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	BROUSSEAU T. ET AL: "(TTA)n repeat polymorphism of the HMG - CoA reductase gene and its association with myocardial infarction and lipoprotein levels: The ECTIM Study." BULLETIN OF MOLECULAR BIOLOGY AND MEDICINE, (1995) 20/1-2 (27-29). , XP000952769 the whole document	1-11
P,X	WO 99 50454 A (WHITEHEAD BIOMEDICAL INST ;LANDER ERIC S (US); CARGILL MICHELE (US) 7 October 1999 (1999-10-07) cited in the application page 1-3; claims; table 1B	1-11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/GB 00/02396

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9845477 A	15-10-1998	AU 6632998 A	30-10-1998
		EP 0972075 A	19-01-2000
WO 9950454 A	07-10-1999	AU 3363899 A	18-10-1999